# VITAMIN DEFICIENCY SYNDROME IN THE ALBINO RAT PRECIPITATED BY CHRONIC ZINC CHLORIDE POISONING*

PAUL GROSS, M.D., ZABOJ HARVALIK, Dr.Sc., AND EDITH RUNNE, A.B., M.A.

The beneficial effect of parenteral liver therapy in arsphenamine reaction and gold dermatitis has been recognized by clinical investigators. Although there may be other detoxifying substances in liver extract, we have been interested in the rôle which the Vitamin B complex plays in the therapeutic action of liver extract. While planning experiments along these lines, we discovered a toxic effect of zinc chloride in rats under certain experimental conditions which promises to shed some light on the mechanism of chronic metallic poisoning.

## EXPERIMENTS

### *Group 1—Controls*

Female albino rats, at ages from 21 to 24 days and weighing around 40 grams, were kept in individual cages and fed the following ration:†

| | |
|---|---|
| Casein (Labco) | 20 |
| Cerelose | 73 |

* From the Department of Dermatology, College of Physicians and Surgeons, Columbia University.

This work was supported in part by a grant from the John and Mary R. Markle Foundation.

Read before the Fourth Annual Meeting of The Society for Investigative Dermatology, Cleveland, Ohio, June 3, 1941.

† The Thiamin chloride, Riboflavin, Pyridoxin, Nicotinic acid and Calcium Pantothenate Dextrorotary, were furnished by Dr. J. M. Carlisle of Merck & Company.

The Ethyl linoleate was made and supplied by Dr. G. E. Martin of the William R. Warner Laboratories for Therapeutic Research, and the Wheat Germ Oil was made available by Dr. Ezra Levin of the Viobin Corporation.

We wish to express our appreciation for these gifts.

| | |
|---|---|
| Salts (Osborn Mendel) | 4 |
| Wheat germ oil | 2 |
| Cod liver oil | 1 |

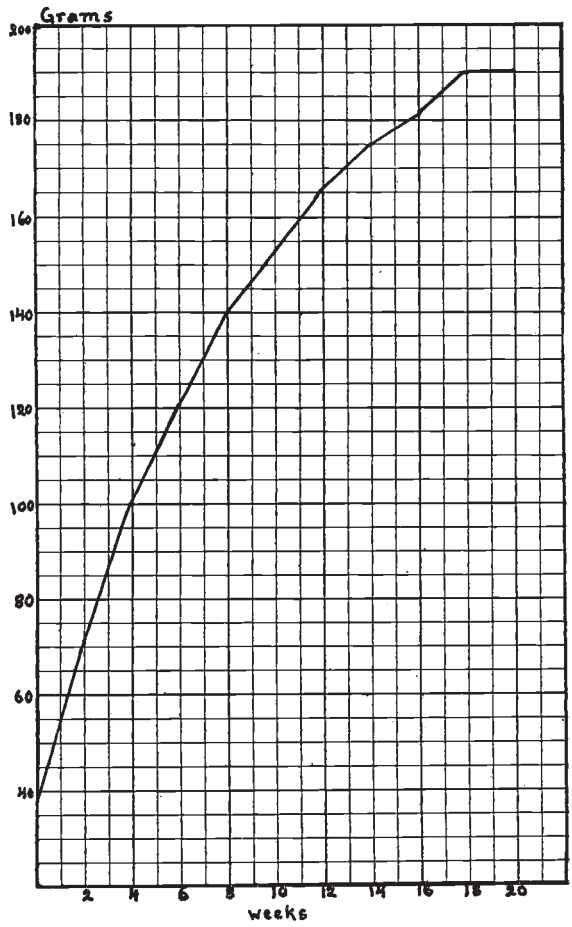


FIG. 1. WEIGHT CURVE OF CONTROL RATS (GROUP 1) RECEIVING A SYNTHETIC DIET SUBOPTIMAL ONLY IN PANTOTHENIC ACID, SUPPLIED BY 100 MGM. OF LABCO RICE POLISH FACTOR II (DIET S.O.)

The supplements consisted of:

| | |
|---|---|
| Choline chloride | 15 mgm. for the first four weeks and 1 mgm. thereafter |

| | |
|---|---|
| Thiamin chloride | 20 micrograms |
| Riboflavin | 50 micrograms |
| Pyridoxine | 40 micrograms |
| Rice polish factor II (Labco) | 100 mgm. (filtrate fraction) |
| Nicotinic acid | 1 mgm. |
| Ethyl Linoleate | 2 drops |

All supplements were fed daily by syringe except on Sunday.

Animals on this diet have a normal appearance but their weight curve does not come up to the standards established for a synthetic diet of the rat.

Supplee (1) and co-workers have shown the effectiveness of the filtrate fraction in Labco rice polish factor II, when added in daily doses of 100 mgm. to a diet similar to ours, and supplemented with thiamin 12.5 micrograms, riboflavin 10 micrograms, pyridoxine 10 micrograms, and Crisco 3 per cent. They found it was adequate for a normal rate of development of about 12 to 15 grams per week. The average weight curve of our control animals compares favorably with those reported by Supplee and co-workers, but the weekly gains are lower than those obtained with a synthetic diet supplemented with optimal amounts of pantothenic acid, according to Unna (2).

Outside of the inferior weight curve and rusting of the otherwise well kept fur, which cleared up spontaneously, control rats observed over a period of 20 weeks showed no skin lesions associated with pantothenic acid deficiency of the albino rat.

We have designated this diet with the initials "S.O." to indicate its suboptimal nature.

*Group 2*

Eight albino rats under the same experimental conditions as Group 1 were fed diet S.O., and four hours after the syringe feeding of supplements, 4 mgm. of zinc chloride dissolved in cod liver oil[1] were instilled into the mouth of the animal by syringe using a simple device to spread the mouth. This was done daily except on Sundays.

Three to five weeks after the beginning of the experiment, five

[1]A saturated solution of zinc chloride in alcohol was mixed with the oil shortly before each feeding.

of the eight animals showed retardation of growth, expressed by a plateau of the weight curve, and various skin manifestations

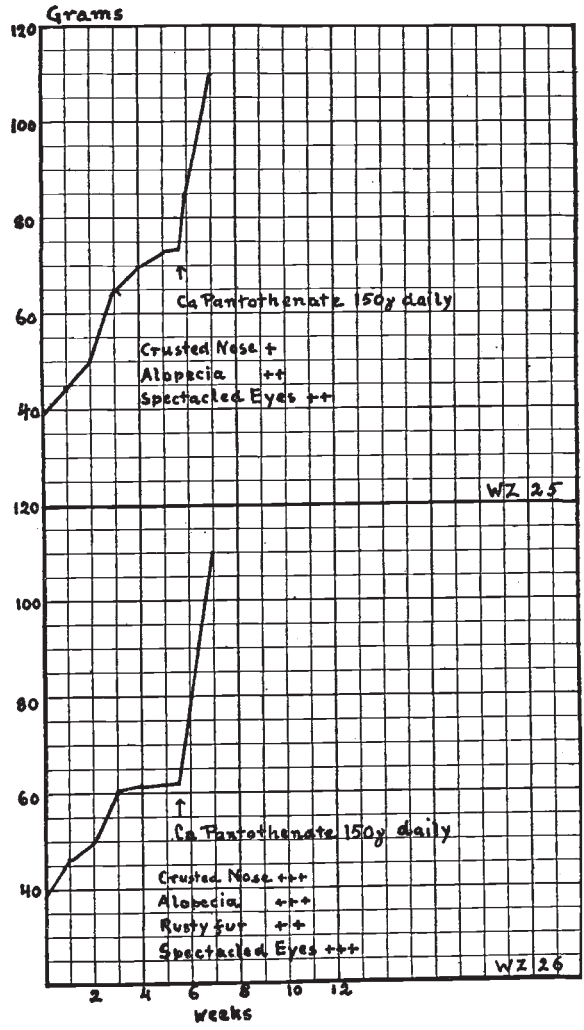


FIG. 2. WEIGHT CURVE OF WHITE RATS (GROUP 2) FED DIET S.O., DEVELOPING A DEFICIENCY AFTER DAILY SYRINGE FEEDING OF 4 MGM. ZINC CHLORIDE
Recovery after addition of 150 microgram calcium pantothenate per day

described by György and coworkers (3), and commonly identified with pantothenic acid deficiency. Four animals developed a

severe alopecia, especially on the face, head, neck, back and belly; three a mild alopecia; five animals showed severe rusting of the fur and a ruffled appearance; four had crusting of the nose and chin; and three animals developed crusting of the eyelids and alopecia, giving the well-known spectacled appearance. One of these animals died five and one-half weeks after the beginning of the experiment.

At the end of the sixth week, the seven surviving animals were fed 150 micrograms of synthetic calcium pantothenate daily in addition to 100 mgm. of the filtrate fraction. Although the zinc chloride feeding was continued at the same level, the animals immediately began to gain weight and showed increases from 12 to 27 grams for the first week. The deficiency symptoms responded promptly and the spectacled eye condition disappeared within ten days and the re-growth of hair was complete in three weeks. No rusting of the fur was noted at the end of this period.

*Group 3*

Six black and eight white rats kept under the same experimental condition and fed diet S.O. received 5 to 6 mgm. of zinc chloride in olive oil, four hours after the feeding of supplements. The black rats developed severe greying of the fur in the fourth to fifth week, and the white rats showed severe rusting. In both groups manifestations of deficiency developed, consisting of crusting of the snout, blood caked whiskers, ruffled and matted fur, with alopecia of varying degree especially on the back of the neck, belly and hind back, redness of the skin, especially on the belly and hind back, in some animals accompanied by scaling; hemorrhagic ears and crusted tails. So far only one animal has developed a spectacled eye condition. This group developed the deficiency later than Group 2, and therefore the weight curves reached higher levels. The experiment is still being continued.

*Group 4*

Twenty-four white rats were fed the diet S.O. under the same experimental conditions, receiving in addition to 100 mgm. of filtrate fraction, a daily dose of 100 micrograms of synthetic cal-

cium pantothenate per day. They were divided into six groups, receiving zinc chloride in oil in amounts varying from 4 to 15 mgm.

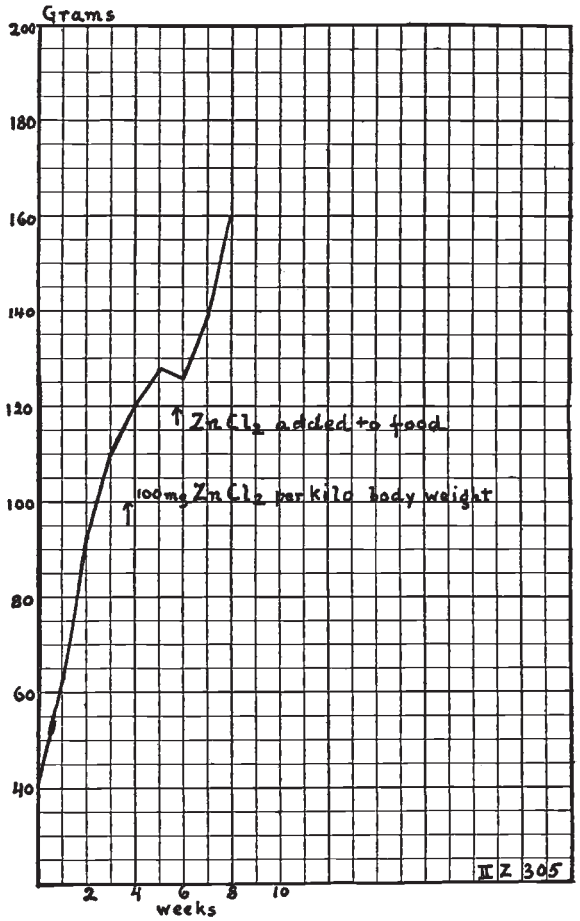


FIG. 3. WEIGHT CURVE OF A WHITE RAT (GROUP 4) FED 100 MICROGRAM CALCIUM PANTOTHENATE DAILY IN ADDITION TO 100 MGM. FILTRATE FRACTION OF DIET S.O.

Zinc chloride fed at 6 mgm. level per day, shows no toxic effect; increase to 100 mgm. zinc chloride per kilo body weight causes weight decrease. Recovery after mixing the same amount of zinc chloride with food.

of zinc chloride per day, fed about seven hours after the vitamins were given. The weight curves of these animals, averaging a gain of 20 grams per week, registered only a slight toxic effect of

zinc chloride. Therefore, the amount of zinc chloride was increased in the fourth week of the experiment so that the animals received 75, 100, 125, 150, 200 and 250 mgm. per kilogram body weight. The weight curves of most of the animals under this regime reached a plateau or declined in the sixth week. No pronounced skin lesions had developed, but the fur of most of the animals was rusted. The feeding of zinc chloride in these larger quantities gave considerable difficulties due to the caustic and nauseating effect of the salt even when dissolved in oil. Therefore, the desired amount of zinc chloride was incorporated in 5 grams of the food mixture which was offered the animals after 16 hours fasting and before the regular food cups were returned to the cages. Some animals refused the food containing the zinc chloride but most animals completely ate up the mixture. This change of experimental conditions produced a prompt increase in weight even in the animals which had eaten the full amount of zinc chloride mixed in food. After it was found that the weight increase improved in the second week of this feeding technic, the experiment was terminated.

## ANALYSIS OF EXPERIMENTS

By feeding a group of rats a diet suboptimal in its content of pantothenic acid but protective against clinical manifestations of pantothenic acid deficiency, it was possible to demonstrate a toxic effect of zinc chloride. This toxic action was visualized by a vitamin deficiency syndrome consisting of retardation of growth, expressed by weight, and skin manifastations which have been known to result from pantothenic acid deficiency. The curative effect of synthetic calcium pantothenate added in amounts of 150 micrograms per day to the ration S.O. further suggests that a pantothenic acid or filtrate fraction deficiency had been precipitated by the feeding of zinc chloride. One is justified to argue that the action of zinc consisted in the destruction of the unsaturation of the ingested essential fatty acids in the intestinal tract.

We raise this question for the following reasons: Animals kept on diet S.O. from which all fatty acids had been removed by eliminating wheat germ oil, cod liver oil and ethyl linoleate,

developed a severe deficiency which is characterized by very poor weight curves, spectacled eye condition, panophthalmia and death within six weeks. Identical symptoms developed if biologically inactive fatty acids were added to this fat free diet. In several of our experiments of this kind we used a condensation product resulting from heating the linoleate in the presence of zinc chloride. Against the inactivation of the unsaturated fatty acids by zinc chloride *in vivo* speaks the fact that 150 micrograms of calcium pantothenate per day had no curative effect on the deficiency

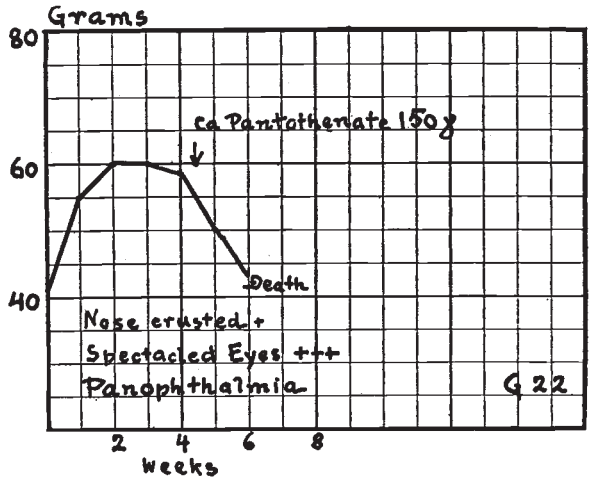


FIG. 4. WEIGHT CURVE OF A WHITE RAT FED A FAT FREE DIET S.O., 150 MICROGRAM CALCIUM PANTOTHENATE PER DAY HAS NO EFFECT ON ADVANCED DEFICIENCY

produced by the fat free diet low in pantothenic acid. The animals thus tested died within a week to ten days with a rapidly declining weight curve. The prompt weight response and the time of cure of the deficiency syndrome as observed in Group 2 of our animals following the feeding of 150 micrograms of pantothenic acid corresponds perfectly to the effect of high pantothenic acid supplements on rats which had developed filtrate fraction deficiency.

The observation that the mixing with the food neutralized the toxic effect of zinc chloride suggests that a chemical combination had been formed with a substance in casein. This would agree with the statement of Eichholtz (4) that milk inhibits the toxic

effect of zinc chloride. The same author also refers to the lipo solubility of zinc chloride which accounts for its high toxicity compared to other zinc salts. We believe that the lipo solubility and higher reactivity of zinc chloride is responsible for the successful demonstration of zinc toxicity in the rat. A caustic effect on the intestinal wall can be ruled out since the animals receiving curative doses of pantothenic acid recovered despite the combined feeding of zinc chloride. It seems more probable that zinc in the form of zinc chloride exerts an action on metabolic processes in the intestinal wall or that the absorption of zinc ions into the portal circulation is higher when zinc chloride is used.

## DISCUSSION

An indication of the toxic effect of prolonged feeding of zinc in rats has been reported by W. R. Sutton (5) who, using zinc carbonate in amounts of 0.5 per cent of the food ration for 39 weeks, observed the following symptoms: Retarded growth to 80 per cent normal weight, diminished food consumption and anemia due to lowered hemoglobin of the erythrocytes which were not diminished in number, loss of fertility in female rats, and greatly increased urine volume in male rats.

Zinc has been proven an essential metal for the nutrition of the rat by Hove, Elvehjem and Hart (6, 7) as well as by Day and McCollum (8). Zinc deficiency, according to these authors, results in the reduction of the alkaline phosphatase of the serum. The action of zinc on various enzymes has been studied in vitro. Hove, Elvehjem and Hart (7) have shown that zinc activates the hydrolysis of sodium hexosediphosphate and sodium pyrophosphate by crude intestinal phosphatase while crude bone phosphatase was inhibited progressively by increasing concentration of the zinc ions. In another publication, the same authors (9) reported on a marked inhibition in vitro of carbonic anhydrase by relatively large amounts of inorganic zinc. The diphasic action of zinc was also shown by Lohman and Kossel (10) in the case of yeast decarboxylase which was inhibited or stimulated according to concentration.

If an enzymatic function is activated by small (physiologic)

amounts of the metal and inhibited by large (toxic) quantities, one would expect similar biological effects from the deficiency of an essential metal as one obtains from its toxic inhibitory action. It is, therefore, not surprising that Day and McCollum (8) described retarded growth, marked eczema, and some alopecia in several of their zinc deficiency animals, a symptomatology closely resembling that of our zinc poisoned rats.

We are now engaged in enzyme studies of rats showing the effect of chronic zinc poisoning and hope to obtain some information on the relationship of pantothenic acid and unsaturated fatty acids to the metabolism of the rat.

Our experimental conditions using suboptimal doses of pantothenic acid have been helpful in demonstrating the biologic effects of chronic zinc chloride poisoning, but have also brought out the importance of an optimal diet for the protection against chronic metal poisoning. They have further suggested that pantothenic acid has a specific curative effect on zinc chloride intoxication.

The prevention of metal poisoning by dietary measures has become a fruitful field since Eisner (11) first proved that green fodder, beets, etc. prevented death in rabbits poisoned by uranil nitrate.

Recent studies on chronic selenium poisoning of the rat by Smith (12, 13) and also by Lewis (14) and co-workers have called attention to the protective action of a high protein diet against the toxicity of seleniferous wheat flour.

## CLINICAL IMPLICATION

Since it has been shown in our experiments that a metallic poisoning can produce a clinically recognizable deficiency disease, one is justified in asking how many of the symptoms due to chronic heavy metal intoxication are subclinical manifestations of vitamin deficiency, produced by the injurious effect of the therapeutic metal used. The varying sites of organic injuries by heavy metals may have a close relation to the resulting impairment of enzymatic functions.

A better knowledge of the nutritional factors which exert a protective action against metallic poisons may eventually be of

help in the successful prevention of arsphenamine and other heavy metal reactions, not based on an allergic mechanism.

Only then will it be possible to place our dietary regulations for the patient receiving anti-syphilitic treatment on a rational basis with regard to the protein-fat-carbohydrate balance and to make the proper selection of the vitamins which offer the best protection against a given heavy metal.

## SUMMARY AND CONCLUSIONS

Young female rats kept on a synthetic diet and a filtrate fraction low in pantothenic acid were fed zinc chloride dissolved in oil in daily doses of 4–6 mgm. by syringe. Most animals developed deficiency symptoms which were completely absent in control animals receiving the identical diet with the omission of zinc chloride.

The deficiency syndrome thus produced in a group of animals could be cured by additional feeding of 150 micrograms of calcium pantothenate per day.

Larger amounts of zinc chloride were necessary to produce a weight plateau in rats fed the same diet with the addition of 100 micrograms of calcium pantothenate.

If zinc chloride was added to 5 grams of the food mixture and fed, its toxic effect was apparently lost.

It is believed that the toxic action of zinc chloride in oil directly introduced into the intestinal tract is due to the higher activity of zinc ions thus fed.

The interaction of zinc with known enzyme systems and the rôle of pantothenic acid are under investigation.

## REFERENCES

(1) Supplee, G. C., Bender, R. C., and Kahlenberg, O. J.: The effect of complementing factors on the quantitative response and specificity of vitamin $B_6$. J. Nutrition, **20:** 109 (Aug.) 1940.

(2) Unna, K.: Pantothenic acid requirement of the rat. J. Nutrition, **20:** 565 (Dec.) 1940.

(3) György, P., Poling, C. E., and Subbarow, Y.: Experiments on the antidermatitis component of the filtrate factor in rats. Proc. Soc. Exper. Biol. & Med., **42:** 738 (Dec.) 1939.

(4) Eichholtz, F.: Zink, in: Handbuch der experimentellen Pharmakologie (Heffter & Heubner), Berlin, Springer, 1934, vol. 3, pt. 3.

(5) SUTTON, W. R.: Some changes produced in growth, reproduction, blood and urine of rats by salts of zinc with certain observations on the effects of cadmium and beryllium salts. Iowa State Coll. J. Sc., **14:** 89 (Oct.) 1939.
(6) HOVE, E., ELVEHJEM, C. A., AND HART, E. B.: Physiology of zinc in nutrition of rat. Am. J. Physiol., **119:** 768 (Aug.) 1937.
(7) HOVE, E., ELVEHJEM, C. A., AND HART, E. B.: The effect of zinc on alkaline phosphatases. J. Biol. Chem., **134:** 425 (June) 1940.
(8) DAY, H. G., AND MCCOLLUM, E. V.: Effects of acute dietary zinc deficiency in the rat. Proc. Soc. Exper. Biol. & Med., **45:** 282 (Oct.) 1940.
(9) HOVE, E., ELVEHJEM, C. A., AND HART, E. B.: The relation of zinc to carbonic anhydrase. J. Biol. Chem., **136:** 425 (Nov.) 1940.
(10) LOHMANN, K., AND KOSSEL, A. J.: Über die Einwirkung des Zinks und anderer Metalle auf die Carboxylase. Naturwissenschaften, **27:** 595 (Sept. 1) 1939.
(11) EISNER, G.: Über die lebensrettende Wirkung von Pflanzenteilen und daraus isolierten Säften bei der tödlich verlaufenden subakuten Uranvergiftung. Biochem. Ztschr., **232:** 218, 1931.
(12) SMITH, M. I.: The influence of diet on the chronic toxicity of selenium. Pub. Health Rep., **54:** 1441 (Aug. 4) 1939.
(13) SMITH, M. I.: Chronic endemic selenium poisoning. A review of the more recent field and laboratory studies. J. A. M. A., **116:** 562 (Feb. 15) 1941.
(14) LEWIS, H. B., SCHULTZ, J., AND GORTNER, R. A., JR.: Dietary protein and the toxicity of sodium selenite in the white rat. J. Pharmacol. & Exper. Therap., **68:** 292 (Feb.) 1940.

## DISCUSSION

DR. PAUL GYORGY, *Cleveland*: My simple excuse for discussing this paper is that the speaker has asked me to comment on his paper with the emphasis on "*kindness.*" It is very easy for me to be "kind" because the paper is a very important contribution to experimental dermatology, and that makes my task even easier, because this paper is "right down my alley" for two reasons: (1) it popularizes experimental dermatology, in which I have been interested for the last 12 years, and (2) it is a very good example of the problem which has lately become very acute—and that is the question whether we are dealing with intoxication in a special pathologic condition or deficiency. I will not go into details. Here, you see, one could, from one point of view describe this condition as zinc intoxication and the production of the disease by the effect of zinc being added to the diet. On the other hand, it is a deficiency, because the effect of zinc chloride can be neutralized. The common denominator is pantothenic acid deficiency. The need for panthothenic acid is greater.

I am reminded in this connection of only one other example where the intoxication versus the deficiency consists of metal and vitamin, that is manganese and vitamin B-1. Perla has shown a few years ago that the addition of manganese or a manganese compound to the diet had a toxic effect on rats, leading to cannibalism, diminished fertility, and by raising the B-1 level the effect of manganese can be neutralized. There is a definite relationship between manganese and thiamin,

vitamin B-1. The explanation may be easily furnished. Vitamin B-1 is very important and necessary for decarbolylation of pyruvic acid, which is in turn, absolutely essential for the metabolism of carbohydrates, and manganese is one of the most important catalytic activators of the system.

I think Dr. Gross is on the right track when he also looks for such an interrelationship in the enzyme system and pantothenic acid.

DR. ARTHUR SCHOCH, *Dallas*: I would like to ask a question and make one observation. It seems to me that the most obvious explanation for the problem just before us is that zinc combines with pantothenic acid to give an insoluble substance. That is so simple and obvious that I hesitate to mention it, but it has not been mentioned at all to date.

I would also like to recount an observation made in our clinical practice for the past six months, and that is that giving large doses, of 40–50 mgm. intravenously has sharply reduced the incidence of gastro-intestinal reactions to pentavalent arsenicals. This has been mainly vomiting, but the same individuals, when given 40–50 mgm. of vitamin B-1, lose all of their gastro-intestinal intolerance to the quinqui and pentavalent drug; this is not true in every case, but in 50 to 75 per cent of our cases this has been found to be true.

DR. LOUIS SCHWARTZ, *Bethesda, Md.*: I realize the great importance which this paper plays in our national defense. If the observations which were made for animals hold for human beings, then it is of great importance in the industrial fields. A great deal of the materials, air planes, etc., being manufactured for national defense require the manufacture of dyes made of zinc and lead alloys, and the industrial engineers are busy devising ventillating systems to prevent zinc and lead poisoning among the workers who make these dyes. If pantothenic acid is given to these people, and has the same effect as it had on the animals, then I think Dr. Gross really deserves a medal of honor.

DR. J. GARDNER HOPKINS, *New York, N. Y.*: This paper has interested me particularly because it throws light on a thing which has been puzzling us for so long—that conditions which we call vitamin deficiencies because they are cured by high vitamin administration, occur in patients whose intake of vitamins seems to have been normal.

Many hypotheses have been presented to explain this phenomenon, such as failure of absorption, and inadequate utilization in the liver. Probably the explanation is not the same in all cases. This work indicates that inactivation of an enzyme by a poison may create a demand for an excessive amount of the vitamins which form that enzyme.

DR. PAUL GROSS, *New York*: I am grateful to Dr. György for his discussion. His reference to manganese and vitamin $B_1$ is very interesting and would run parallel to our findings. On the other hand, we know that manganese deficiency leads to graying of hair in rats.

In answer to Dr. Schoch's question, zinc has no destructive effect on pantothenic acid. Actually, zinc pantothenate has been used in the early experiments and found to be an effective supplement. As far as the beneficial effect of liver

therapy in arsphenamine reactions is concerned, we cannot definitely state the factor in the B-complex responsible for the effect. That detoxication is not a function of vitamins only has been demonstrated recently by Martin and Thompson, especially for amino acids.

It was very gratifying to hear the remarks of Dr. Schwartz and we hope that our work will eventually lead to a rational prophylaxis against chronic metallic poisoning in industry.

I am obliged to Dr. Hopkins for the support given by his discussion and particularly for the encouragement offered to us throughout the whole experiment.